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(71) Applicant (for all designated States except US):
ACADEMISCH ZIEKENHUIS BIJ DE UNIVER-
SITEIT VAN AMSTERDAM [NL/NL]; Meibergdreef 9,
NL-1105 AZ Amsterdam-Zuiddreef (NL).

(72) Inventors; and

(75) Inventors/Applicants (for US only): PEPPELEN-
BOSCH, Maikel, Petrus [NL/NL]; Obrechtstraat
74, NL-3572 EH Utrecht (NL). ZIVKOVIC, Danica
[NL/NL]; Ramstraat 39, NL-3581 HE Utrecht (NL).

DIKS, Sander [NL/NL]; Smalle haven 57, NL-5211 TJ
'S-Hertogenbosch (NL). BINK, Robert, Jozef [NL/NL];
Louis Armstrongkade 176, NL-2551 WV 'S-Gravenhage
(NL).

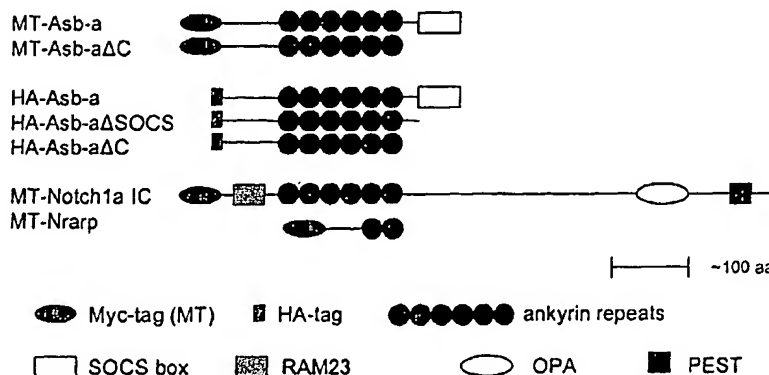
(74) Agent: VAN WESTENBRUGGE, ANDRIES et al.;
Nederlandsch Octrooibureau, Scheveningsseweg 82, P.O.
Box 29720, NL-2502 LS The Hague (NL).

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SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA,
GN, GQ, GW, ML, MR, NE, SN, TD, TG).

[Continued on next page]

(54) Title: ASB-A TRANSCRIPTION REPRESSOR PROTEINS AND NUCLEIC ACIDS AND THEIR APPLICATION IN EX-
PANSION OF STEM CELLS



(57) Abstract: The present invention relates to methods for expansion of stem or progenitor cells. These methods rely on Asb-a polypeptides or nucleic acids to temporarily suppress differentiation of the cells, thus allowing proliferation and self-renewal of the stem or progenitor cells. Asb-a polypeptides and coding sequences define a class of polypeptides and nucleic acids that are both structurally and functionally highly conserved among vertebrates. Asb-a polypeptides contain 6 ankyrin repeats and a SOCS box that mediate the effect of the polypeptide on the regulation of specific subsets of genes involved in differentiation. The invention discloses various methods to increase the intracellular concentration of an Asb-a polypeptide for suppression of terminal differentiation of the stem or progenitor cells. The invention further relates to Asb-a polypeptides and nucleic acids, vectors and host cells for use in methods for their production and for use in the method for expansion of stem or progenitor cells, as well as to stem or progenitor cells containing exogenous Asb-a polypeptides and nucleic acids.

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INTERNATIONAL SEARCH REPORT

Application No

PCT/NL 03/00831

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N5/06 C07K14/46 C07K14/435

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, EMBL, WPI Data, PAJ, MEDLINE, EMBASE, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|--|-----------------------|
| X | WO 01/98456 A (JAPAN TOBACCO INC ;GETZENBERG ROBERT H (US); GENE LOGIC INC (US);) 27 December 2001 (2001-12-27) see JT460914 protein/nucleic acid which is identical to the human Asb-a protein/nucleic acid and shows 49.8% identity with SEQ ID NO 1 | 17-20, 23,24 |
| X | KILE BENJAMIN T ET AL: "Functional analysis of Asb-1 using genetic modification in mice." MOLECULAR AND CELLULAR BIOLOGY, vol. 21, no. 18, September 2001 (2001-09), pages 6189-6197, XP002238792 ISSN: 0270-7306 mouse Asb-5, shows 93.6% identity with human Asb-a (SEQ ID NO 3) ----- -/- | 17-20, 23,24 |

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
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- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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- *G* document member of the same patent family

Date of the actual completion of the international search

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Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel (+31-70) 340-2040, Tx 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Lanzrein, M

INTERNATIONAL SEARCH REPORT

Application No

PCT/NL 03/00831

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|--|-----------------------|
| X | <p>WO 01/96375 A (GLUCKSMANN MARIA ALEXENDRA ;KADAMBI VIVEK JAIRAM (US); MILLENNIUM) 20 December 2001 (2001-12-20) seq id no 2 (claim 13; Fig. 1B; p. 115) encodes human c/SKARP-1 which has 48.6% identity with SEQ ID NO 1; and 53.8 identity with SEQ ID NO 3 and corresponding nucleic acid sequences.</p> | 17-20, 23,24 |
| X | <p>DATABASE EMBL 'Online! 8 February 2001 (2001-02-08), XP002238793 retrieved from EBI Database accession no. AK004396 56,8% identity over 834 nt with SEQ ID NO 2 abstract</p> | 17-20, 23,24 |
| X | <p>DATABASE EMBL 'Online! 6 June 2002 (2002-06-06), XP002238794 retrieved from EBI Database accession no. AL743766 98.7% identity with SEQ ID NO 2 over 160 nt abstract</p> | 17-20, 23,24 |
| X | <p>DATABASE EMBL 'Online! 21 October 2001 (2001-10-21), XP002238795 retrieved from EBI Database accession no. BI891687 97% identity with SEQ ID NO 2 over 577 nt. abstract</p> | 17-20, 23,24 |
| X | <p>DATABASE EMBL 'Online! 16 July 2002 (2002-07-16), XP002238796 retrieved from EBI Database accession no. AK096441 99.8% identity over 990 nt (full length) with SEQ ID NO 4. abstract</p> | 17-20, 23,24 |
| X | <p>DATABASE EMBL 'Online! XP002238797 retrieved from EBI Database accession no. AY057053 100% identity over 990 nt (full length) with SEQ ID NO 4 abstract</p> | 17-20, 23,24 |

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INTERNATIONAL SEARCH REPORT

Application No

PCT/NL 03/00831

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|---|-----------------------|
| A | <p>KILE B T ET AL: "Cloning and characterization of the genes encoding the ankyrin repeat and SOCS box-containing proteins Asb-1, Asb-2, Asb-3 and Asb-4" GENE, ELSEVIER BIOMEDICAL PRESS. AMSTERDAM, NL, vol. 258, no. 1-2, 27 November 2000 (2000-11-27), pages 31-41, XP004223746 ISSN: 0378-1119</p> <p>-----</p> | |
| A | <p>SVENDSEN C N ET AL: "A NEW METHOD FOR THE RAPID AND LONG TERM GROWTH OF HUMAN NEURAL PRECURSOR CELLS" JOURNAL OF NEUROSCIENCE METHODS, ELSEVIER SCIENCE PUBLISHER B.V., AMSTERDAM, NL, vol. 85, 1998, pages 141-152, XP002902582 ISSN: 0165-0270</p> <p>-----</p> | |
| A | <p>ROSE T M ET AL: "ONCOSTATIN M (OSM) INHIBITS THE DIFFERENTIATION OF PLURIPOTENT EMBRYONIC STEM CELLS IN VITRO" CYTOKINE, ACADEMIC PRESS LTD, PHILADELPHIA, PA, US, vol. 6, no. 1, 1994, pages 48-54, XP002912249 ISSN: 1043-4666</p> <p>-----</p> | |
| A | <p>CONOVER J C ET AL: "CILARY NEUROTROPHIC FACTOR MAINTAINS THE PLURIPOTENTIALITY OF EMBRYONIC STEM CELLS" DEVELOPMENT, COMPANY OF BIOLOGISTS, CAMBRIDGE,, GB, vol. 119, 1993, pages 559-565, XP002912248 ISSN: 0950-1991</p> <p>-----</p> | |

INTERNATIONAL SEARCH REPORT

application No.
PCT/NL 03/00831

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this International application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/SA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-16

Method for in vitro expansion of mammalian cells or progenitor cells utilizing an Asb-a polypeptide, fusion proteins thereof or nucleic acid encoding said polypeptide.

2. claims: 17-25 (partially)

An Asb-a polypeptide having an amino acid sequence with at least 39 % amino acid identity with SEQ ID NO: 1, nucleic acid thereof having at least 35% identity with a nucleotide sequence depicted in SEQ ID NO: 2, expression vectors containing said nucleic acid molecule host cell comprising said vector, methods of producing said polypeptide,

3. claims: 17-25 (partially)

An Asb-a polypeptide having an amino acid sequence with at least 39 % amino acid identity with SEQ ID NO: 3, nucleic acid thereof having at least 35% identity with a nucleotide sequence depicted in SEQ ID NO: 4, expression vectors containing said nucleic acid molecule host cell comprising said vector, methods of producing said polypeptide,

4. claims: claims 26-28

Stem cell or progenitor cell comprising an exogenous Asb-a polypeptide, an exogenous nucleotide sequence encoding an Asb-a polypeptide or both, pharmaceutical composition thereof.

INTERNATIONAL SEARCH REPORT

Application No

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| Patent document cited in search report | | Publication date | Patent family member(s) | Publication date |
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| WO 0198456 | A | 27-12-2001 | AU 6844201 A WO 0198456 A2 | 02-01-2002 27-12-2001 |
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